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Genotypic variation in common bean in response to zinc deficiency in calcareous soil

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Abstract

Greenhouse experiments have been carried out to study the genotypic variation among 35 bean (*Phaseolus vulgaris* L.) genotypes with regards to tolerance to zinc (Zn) deficiency (Zn efficiency). Plants were grown for 45 days in Zn deficient soil supplemented with 0 or 5 μg Zn g^{-1} soil) and analyzed for Zn efficiency, plant Zn concentration and content, and the distribution of Zn between old and young parts of the shoot. Zn efficiency (ZE) was defined as the ratio of dry matter production at low and high Zn supply and was calculated for the whole shoot as well as for young and old parts of the shoot. There were marked differences in ZE among the bean genotypes. Genotypes G4449 and G11360 were about 2-fold and 10-fold more Zn-efficient than G11229 and G3871 in whole shoot and young-part based ZE, respectively. Interestingly, the older portions of the shoot for most genotypes had higher dry matter production under Zn deficiency than under sufficient Zn supply, suggesting that there was a significant inhibition of new shoot growth and transport of photosynthates from source to sink organs under low-Zn conditions. Zinc concentrations of both old and young portions of the shoot did not correlate with ZE, but shoot Zn content was found to be significantly correlated with ZE. Furthermore, Zn-efficient genotypes distributed more Zn into young parts of the shoot under Zn-deficient conditions than did the inefficient lines. Variation in seed Zn content did not significantly influence the determination of ZE. We concluded that there is a substantial variation in Zn efficiency in the bean genome, and ZE based on analysis of the young shoot tissues represents a suitable screening technique for the evaluation of ZE in low-Zn soils.

Introduction

Zinc deficiency is a global nutritional problem in crop production. Thirty per cent of the world soils are Zn deficient, including many agricultural lands in Turkey, India and Australia (Cakmak et al., 1999; Hacisalihoglu and Kochian, 2003; Rengel, 2001). Correction of Zn deficiency via fertilization is not always the ideal solution because of the influence of agronomic and economic factors including reduced Zn availability in dry topsoil, subsoil constraints, disease interactions, and the high relative cost of fertilizer in developing countries (Graham and Rengel, 1993). Therefore, identification and cultivation of Zn-efficient genotypes that could use soil or tissue Zn efficiently is a realistic alternative to Zn fertilizer application in some edaphic environments. Differential ZE has been reported in several crop species including common bean (*Phaseolus vulgaris* L.) (Ambler and Brown, 1969; Singh and Westermann, 2002) and wheat (Cakmak et al., 1997; 1998; Rengel and Romheld, 2000). Despite its complexity, there is substantial interest in ZE. Understanding the mechanisms of ZE can greatly con-

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tribute to the selection and breeding of genotypes with higher tolerance to Zn-deficient soils. Some progress has been made in understanding the physiological and biochemical mechanisms of this trait (Cakmak et al., 1998; Erenoglu et al., 1999; Grotz et al., 1998; Hacisalihoglu and Kochian, 2003; Hacisalihoglu et al., 2001, 2003a, b; Kochian, 1991; Rengel, 2001; Welch, 1995). However, most of these studies have been conducted using cereals, especially wheat. Studies with bean are less common and carried out using only a few genotypes. Therefore, we studied beans using 35 genotypes to collect more reliable information on the extend of genotypic variation in ZE. Development of new bean genotypes with both high tolerance to soil-Zn deficiency conditions and high concentrations of seed-Zn is of high priority because Zn deficiency is a global nutritional problem in soils and human beings (Welch and Graham, 2002; Cakmak, 2002).

Common bean (*Phaseolus vulgaris* L.) is an important staple food crop with a short growing cycle known to be highly sensitive to Zn deficient soils especially under high light intensity (Marschner and Cakmak, 1989). Previous studies have shown that certain bean varieties differed in their tolerance to low Zn supply (Viets et al., 1954; Judy et al., 1965; Moraghan and Grafton, 1999). Polson (1968) found that Saginaw (Zn-efficient) and Sanilac (Zn-inefficient) were valuable navy bean genotypes for studying high-Zn or low-Zn stress tolerance. Very recently, Singh and Westermann (2002) reported that a single dominant gene determines the expression of high tolerance to Zn deficiency in common bean when grown under low soil-Zn conditions. Moreover, Moraghan and Grafton (1999) compared the growth and seed-Zn accumulation of four bean cultivars. They reported that seed-Zn content could be used as an important indicator for selecting Zn-efficient bean genotypes. In studies with wheat cultivars, it was found that shoot-Zn concentrations are not a reliable parameter for screening genotypes for ZE, but genotypic differences in Zn translocation capacity from older into younger organs may be an important factor in the expression of high ZE (Torun et al., 2000).

The objectives of this study were: (1) to characterize the genotypic variability of the Zn efficiency trait in common bean, (2) to identify the most Zn-efficient and –inefficient bean genotype(s) to be used in further genetic studies, (3) to determine the role of concentration and distribution of Zn in old and young shoot parts in the expression of differential ZE, and (4) to examine the contribution of seed mass and seed-Zn concentration to differential ZE.

Materials and methods

Seeds of the common bean genotypes (*Phaseolus vulgaris* L.) used in the present study were obtained from Drs M. Blair and S. Beebe (CIAT), Dr D. Garvin (USDA-ARS, University of Minnesota) and Dr J. Kelly (Michigan State University). To understand the role of seed-Zn concentrations in expression of Zn efficiency, Zn concentrations of bean seeds were determined after digestion of dry seeds in concentrated HNO_3 overnight at 120 °C followed by further digestion in HNO_3: $HClO_4$ (1:1, v/v) at 220 °C. The digestate was diluted with 5% HNO_3 and analyzed for Zn via simultaneous inductively coupled argon-plasma emission spectrometry (ICAP 61E Trace analyzer, Thermo-Jarrel Ashe, Franklin, MA, U.S.A.).

Plants were grown in a greenhouse in Adana, Turkey from October until mid December, 2002. The temperature within the greenhouse was 25 ± 2 °C during the day and 19 ± 2 °C during the night. Plants were grown under natural day length and light intensity. Growth conditions were as described in Cakmak et al. (1997) with some modifications. Briefly, five seeds were sown in plastic pots filled with 2.2 kg of Zn-deficient soil from the Central Anatolia region in Turkey. The soil characteristics were: pH 8.0, $CaCO_3$ 149 g kg^{-1}, organic matter 7 g kg^{-1}, and DTPA-extractable concentrations of Fe, 2.2 μg g^{-1}; Mn, 3.6 μg g^{-1}; Cu, 0.7 μg g^{-1}, and Zn, 0.09 μg g^{-1}. Plants were grown in a greenhouse at two soil-Zn levels [low Zn ($-$Zn = 0) and adequate Zn ($+$Zn = 5 μg g^{-1})] supplied in the form of $ZnSO_4$ together with a basal treatment of 200 μg g^{-1} N, as $Ca(NO_3)_2$ and 100 μg g^{-1} P as KH_2PO_4. After emergence, plants were thinned to three seedlings per pot and watered with deionized water daily, and all pots were randomized every five to six days. Visual Zn deficiency symptoms were seen 25 to 30 days after planting to Zn-deficient soils. Following 45 days of growth, shoots were harvested, and separated into young and old parts. Old parts represent primary leaves and stem parts below the primary leaves; the rest of shoot was designated as young part. After harvesting, plant tissues were dried at 70 °C for 2 d, weighed, ground, dry-ashed at 500 °C; the ash was dissolved in 3.3% HCl, and analyzed for Zn by ICP-AES (Jobin Yvon, Paris). Zinc efficiency (ZE) was calculated for the

whole shoot, old parts and young parts of shoots by considering their dry matter yield as follows:

$$\text{ZE (\%)} = [\text{dry matter yield at } -\text{Zn/dry matter yield at } +\text{Zn}] \times 100$$

The experiments were set up in a complete randomized design with four replicates, and the variation within means is presented as the standard error. Results were analyzed by analysis of variance using seed-Zn as a covariate (ANCOVA) to eliminate experimental error due to differences in seed-Zn content. The differences between means were compared by the Tukey's Honestly Significant Differences (HSD) test at the 5% level of probability.

Results

Zn-deficiency symptoms

Zinc deficiency symptoms, such as interveinal chlorosis on older leaves, shortening of internodes and stunting of plants, appeared as early as 25 to 30 days in plants grown without Zn supplied. Bean genotypes showed marked differences in the severity of visual symptoms displayed. The symptoms were particularly severe in the genotypes G11229, G3871, G734 and G18249, and very slight on other genotypes such as G4449, G11360, G753 and G9975 under Zn deficiency conditions. At adequate Zn supply (5 μg Zn g^{-1} soil), all bean genotypes grew well without the occurrence of visible symptoms.

Dry matter production and Zn efficiency

As expected, adequate Zn supply (+Zn) enhanced whole shoot dry matter production of bean genotypes compared to plants grown under low-Zn conditions, with exception of the genotype G4449 (Table 1). In all Tables, the genotypes are arranged in order of their ZE (from highest to the lowest), based on whole shoot dry weight (Tables 1, 2, 3, 4, and 5). The growth of G4449 was not affected by Zn supply indicating very high tolerance of this genotype to the Zn deficient soil. When only young parts of genotypes were considered, there were marked differences in dry matter production between genotypes under Zn deficiency (Table 1). Most of the Zn-inefficient genotypes showed decreases in dry matter production of young parts caused by Zn deficiency. These differences were 4- to 10-fold in magnitude, but in most of the Zn-efficient genotypes these differences were less than

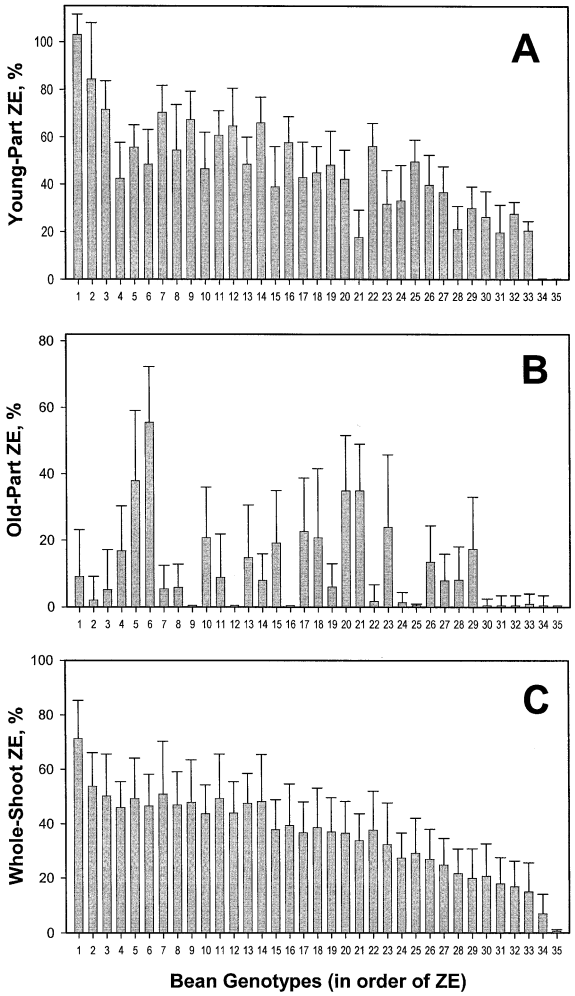

Figure 1. Least squares means (LSMEANS) of Zn efficiency (ZE) of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supply. (A) young shoot part ZE, (B) old shoot part ZE, and (C) whole shoot ZE. LSMEANS were obtained from analysis of covariance using seed Zn content as covariate to minimize the effect of seed Zn on ZE. Vertical bars represent standard error, $n = 4$. Genotypes are listed in order of ZE.

2-fold (Table 1). Interestingly, in all genotypes (except A686) dry matter yield of old parts was greater under Zn deficiency (Table 1). These results may indicate inhibition of photosynthate transport from source to sink tissues resulting from Zn deficiency. The genotypic ZE (as a %; calculated by dividing dry matter yield measured for −Zn plants by that found for +Zn plants) showed a very large variation between genotypes, particularly in the case of young genotype parts (Table 1; Figure 1A-C). The ZE varied between 8.4% (G3971) to 89.6% (G4449) for young parts and from

Table 1. Dry matter values of whole shoot, young and old shoot parts of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. Data are presented as means±SE, $n = 4$ replicates. All genotypes are ranked according to their whole-shoot ZE

Genotype	Whole shoot			Young part			Old part		
	−Zn	+Zn	ZE	−Zn	+Zn	ZE	−Zn	+Zn	ZE
	(mg $plant^{-1}$)		(%)	(mg $plant^{-1}$)		(%)	(mg $plant^{-1}$)		(%)
1. G4449	1266 ± 86	1240 ± 111	102	598 ± 95	667 ± 60	89.6	669 ± 13	573 ± 52	117
2. G11360	1386 ± 61	1511 ± 146	91.8	607 ± 26	859 ± 101	70.7	779 ± 37	651 ± 47	120
3. G12778	1184 ± 103	1376 ± 106	86.1	585 ± 79	875 ± 103	66.8	599 ± 31	500 ± 30	120
4. G753	912 ± 55	1065 ± 163	85.6	262 ± 56	596 ± 158	43.8	650 ± 43	468 ± 30	139
5. G9975	1443 ± 10	1690 ± 202	85.4	590 ± 12	1130 ± 159	52.2	854 ± 21	560 ± 52	153
6. NB585	1029 ± 23	1207 ± 77	85.3	428 ± 21	859 ± 42	49.8	601 ± 7	347 ± 35	173
7. LRK31	1330 ± 43	1566 ± 115	84.9	566 ± 46	913 ± 109	62.0	763 ± 21	653 ± 12	117
8. G3645	848 ± 70	1001 ± 208	84.7	306 ± 52	561 ± 109	54.5	542 ± 24	440 ± 38	123
9. G5285	1196 ± 102	1418 ± 48	84.3	492 ± 45	782 ± 86	62.9	703 ± 58	636 ± 39	111
10. G3096	1096 ± 48	1311 ± 73	83.6	407 ± 21	821 ± 67	49.6	689 ± 44	490 ± 8	141
11. G22415	1337 ± 168	1602 ± 221	83.5	493 ± 76	904 ± 128	54.5	845 ± 117	698 ± 92	121
12. G19048	1158 ± 48	1395 ± 47	83.0	509 ± 16	771 ± 41	66.0	649 ± 37	624 ± 11	104
13. G19142	766 ± 144	929 ± 225	82.4	250 ± 63	534 ± 176	46.8	516 ± 101	395 ± 57	131
14. G21242	1206 ± 95	1465 ± 197	82.3	527 ± 112	903 ± 158	58.3	679 ± 17	562 ± 40	121
15. Ica Pijao	906 ± 105	1192 ± 165	76.0	296 ± 62	752 ± 130	39.4	609 ± 63	441 ± 46	138
16. G11708	1318 ± 131	1734 ± 11	76.0	569 ± 89	1048 ± 6	54.2	749 ± 43	685 ± 2	109
17. G11350	996 ± 51	1313 ± 97	75.9	392 ± 8	873 ± 35	44.9	604 ± 44	441 ± 98	137
18. DR Kid	1060 ± 14	1402 ± 40	75.6	385 ± 99	909 ± 48	42.4	675 ± 90	493 ± 44	137
19. G169	1064 ± 24	1412 ± 79	75.4	434 ± 22	903 ± 65	48.1	630 ± 37	509 ± 14	124
20. G2606	955 ± 68	1278 ± 135	74.7	390 ± 36	906 ± 104	43.0	566 ± 35	372 ± 36	152
21. G7843	720 ± 91	967 ± 77	74.5	133 ± 28	591 ± 67	22.5	587 ± 71	376 ± 12	156
22. G16130	1238 ± 32	1669 ± 181	74.2	531 ± 4	1063 ± 132	49.9	707 ± 27	605 ± 58	117
23. G10060	1000 ± 78	1455 ± 136	68.7	261 ± 65	923 ± 118	28.3	739 ± 14	533 ± 35	139
24. BAT93	701 ± 24	1022 ± 67	68.6	261 ± 51	663 ± 40	39.3	441 ± 35	359 ± 29	123
25. A686	823 ± 69	1222 ± 60	67.3	377 ± 36	766 ± 36	49.2	446 ± 39	456 ± 24	98
26. G87	825 ± 34	1246 ± 105	66.3	342 ± 48	879 ± 48	38.9	483 ± 51	367 ± 59	132
27. Saginaw	692 ± 38	1065 ± 109	65.0	319 ± 5	775 ± 96	41.1	373 ± 42	290 ± 13	129
28. G1934	832 ± 41	1332 ± 175	62.5	242 ± 23	877 ± 132	27.6	590 ± 23	455 ± 43	130
29. G11656	707 ± 76	1190 ± 81	59.4	283 ± 70	879 ± 90	32.2	424 ± 18	311 ± 21	137
30. G5034	1027 ± 86	1739 ± 135	59.1	294 ± 47	1091 ± 104	26.9	733 ± 45	648 ± 36	113
31. G18249	807 ± 92	1375 ± 162	58.7	214 ± 66	849 ± 141	25.2	594 ± 30	526 ± 26	113
32. Sanilac	565 ± 9	970 ± 28	58.2	230 ± 7	686 ± 28	33.5	335 ± 8	284 ± 16	118
33. G734	750 ± 22	1372 ± 60	54.6	219 ± 13	931 ± 39	23.5	531 ± 16	441 ± 30	120
34. G3971	484 ± 50	978 ± 64	49.5	53 ± 12	627 ± 47	8.4	432 ± 44	351 ± 21	123
35. G11229	485 ± 93	1138 ± 157	42.6	70 ± 35	798 ± 113	8.8	414 ± 58	340 ± 56	122
Tukey's $HSD_{0.05}$	247	417	36	176	331	35	154	337	51

42.6% (G11229) to 102% (G4449) for whole shoot. In the case of old genotype parts, ZE values were above 100%, except for genotype A686 (Table 1).

Zn concentration and content

The Zn concentrations in young and old parts of the shoot of −Zn plants were very low, ranging from 5.5 to 7.3 μg/g for young parts and from 6.0 to 11.5 μg/g for old parts (Table 2). Overall, Zn deficiency decreased shoot Zn concentrations 80% in old parts and 60% in

Table 2. Shoot-Zn concentrations of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. Data are presented as means ± S.E., $n = 4$ replicates. All genotypes are ranked according to their whole-shoot ZE

Genotype		−Zn		+Zn	
		Young part	Old part	Young part	Old part
	ZE%	μg Zn g^{-1}			
1. G4449	102	6.93 ± 0.64	7.80 ± 2.46	23.5 ± 2.81	21.7 ± 1.92
2. G11360	92	5.47 ± 0.46	7.18 ± 0.35	31.1 ± 1.45	18.1 ± 0.76
3. G12778	86	7.13 ± 0.29	11.1 ± 0.79	36.9 ± 0.87	24.1 ± 2.33
4. G753	86	5.44 ± 0.19	6.02 ± 0.12	35.8 ± 1.55	18.1 ± 1.81
5. G9975	85	6.49 ± 0.58	8.74 ± 1.00	26.3 ± 1.22	18.9 ± 2.49
6. NB585	85	6.54 ± 0.24	8.37 ± 0.23	33.9 ± 1.71	17.5 ± 0.21
7. LRK31	85	7.15 ± 0.59	11.4 ± 0.71	26.4 ± 1.81	18.8 ± 1.58
8. G3645	85	6.38 ± 0.52	8.29 ± 0.19	40.9 ± 2.69	25.3 ± 2.19
9. G5285	84	6.01 ± 0.66	9.46 ± 0.24	35.9 ± 2.30	23.7 ± 2.29
10. G3096	84	6.23 ± 0.45	8.27 ± 0.46	33.0 ± 0.11	18.8 ± 1.11
11. G22415	84	6.83 ± 0.25	10.3 ± 0.25	26.8 ± 1.23	19.1 ± 0.78
12. G19048	83	6.44 ± 0.01	7.99 ± 0.59	31.3 ± 3.03	19.8 ± 1.52
13. G19142	82	5.73 ± 0.06	8.59 ± 1.00	31.7 ± 2.88	18.9 ± 1.75
14. G21242	82	6.85 ± 0.51	9.98 ± 0.59	30.3 ± 1.47	18.4 ± 0.98
15. Ica Pijao	76	5.90 ± 0.69	7.76 ± 0.99	37.0 ± 2.66	21.1 ± 2.08
16. G11708	76	6.56 ± 0.23	11.3 ± 0.39	27.9 ± 0.39	20.9 ± 0.79
17. G11350	76	6.81 ± 0.88	6.99 ± 0.67	30.1 ± 1.14	17.9 ± 0.12
18. DR Kidney	76	7.30 ± 0.27	9.78 ± 0.85	27.6 ± 1.33	17.7 ± 1.24
19. G169	75	6.36 ± 0.72	8.84 ± 0.52	38.2 ± 1.14	29.4 ± 0.91
20. G2606	75	6.94 ± 0.45	7.60 ± 0.28	35.0 ± 1.71	19.4 ± 1.71
21. G7843	75	7.36 ± 0.27	7.50 ± 0.30	34.8 ± 0.87	19.8 ± 1.92
22. G16130	74	6.00 ± 0.30	9.69 ± 0.13	26.7 ± 1.50	19.3 ± 0.30
23. G10060	69	7.33 ± 0.03	7.95 ± 0.58	25.1 ± 1.08	16.4 ± 0.90
24. BAT93	69	5.59 ± 0.25	8.48 ± 0.46	34.9 ± 0.18	24.4 ± 1.32
25. A686	67	6.39 ± 0.16	11.5 ± 0.60	33.4 ± 3.24	24.1 ± 0.67
26. G87	66	6.63 ± 0.46	9.45 ± 0.31	42.5 ± 0.70	25.7 ± 2.66
27. Saginaw	65	6.94 ± 0.30	10.4 ± 0.42	39.5 ± 2.80	22.4 ± 2.67
28. G1934	63	6.48 ± 0.32	6.73 ± 0.36	34.3 ± 0.83	18.4 ± 0.01
29. G11656A	59	6.49 ± 0.28	7.75 ± 0.09	31.0 ± 0.47	14.7 ± 0.71
30. G5034	59	6.19 ± 0.15	7.80 ± 0.46	23.5 ± 2.81	15.4 ± 0.91
31. G18249	59	7.17 ± 0.71	8.17 ± 0.64	40.3 ± 1.26	23.2 ± 0.72
32. Sanilac	58	7.27 ± 0.73	9.32 ± 0.45	36.5 ± 3.75	20.7 ± 1.55
33. G734	55	6.80 ± 0.13	7.70 ± 0.30	40.5 ± 2.37	21.5 ± 1.28
34. G3971	50	6.56 ± 0.29	6.42 ± 0.38	38.4 ± 1.11	18.3 ± 1.16
35. G11229	43	6.27 ± 0.45	7.12 ± 0.36	31.8 ± 1.74	16.9 ± 1.54
Tukey's HSD0.05		1.45	1.77	6.20	4.98

young parts, when averaged for all genotypes. Despite the substantial variation in ZE between genotypes, Zn concentrations of genotypes did not show a corresponding variation when grown without adequate Zn supply. Zn-efficient and Zn-inefficient genotypes had similar Zn concentrations (Table 2). Consequently, Zn concentrations of old and young parts did not correlate with ZE of the genotypes (Figure 2A–D).

In contrast to Zn concentrations, total Zn content in young parts of the shoot was closely related to ZE (Table 3; Figure 2E–F). For example, under −Zn conditions, the most Zn-efficient and Zn-inefficient genotypes did not exhibit statistically significant dif-

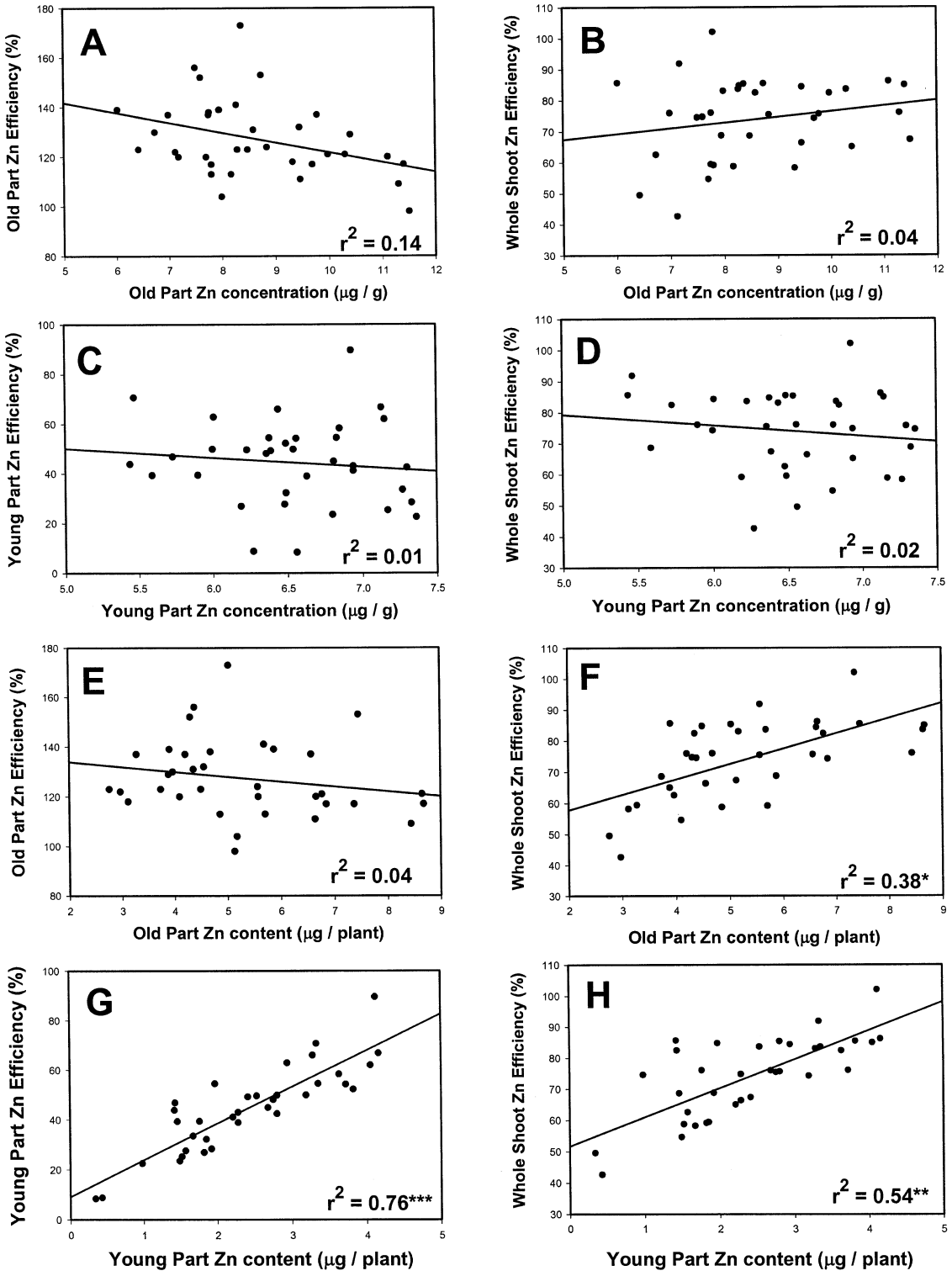

Figure 2. Correlations between % Zn efficiency (ZE) and Zn content or concentration in old parts, young parts or whole shoot of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. For more details, see methods section. *, **, and *** are statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels, respectively, as determined using simple linear regression (solid line is the calculated linear regression line); r^2 = linear regression coefficient squared.

Table 3. The total amount of Zn (Zn content) per young parts, old parts and whole shoot of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. Data are presented as means ± S.E., $n = 4$ replicates. All genotypes are ranked according to their whole-shoot ZE

Genotype		−Zn			+Zn		
		Young part	Old part	Whole shoot	Young part	Old part	Whole shoot
	ZE%	μg Zn $plant^{-1}$					
1. G4449	102	4.12 ± 0.59	7.37 ± 0.41	11.5 ± 0.93	25.0 ± 1.22	12.5 ± 2.08	37.5 ± 2.86
2. G11360	92	3.33 ± 0.42	5.58 ± 0.05	8.91 ± 0.40	26.8 ± 4.18	11.8 ± 1.28	38.6 ± 5.46
3. G12778	86	4.16 ± 0.51	6.66 ± 0.24	10.8 ± 0.28	32.3 ± 3.83	12.0 ± 1.07	44.4 ± 2.87
4. G753	86	1.42 ± 0.26	3.91 ± 0.18	5.33 ± 0.29	21.3 ± 5.82	8.49 ± 1.35	29.8 ± 6.76
5. G9975	85	3.82 ± 0.27	7.46 ± 0.87	11.3 ± 1.11	29.7 ± 4.42	10.6 ± 1.92	40.3 ± 5.39
6. NB585	85	2.80 ± 0.14	5.04 ± 0.19	7.84 ± 0.12	29.2 ± 2.50	6.07 ± 0.54	35.3 ± 3.02
7. LRK31	85	4.05 ± 0.21	8.68 ± 0.34	12.7 ± 0.85	24.2 ± 1.81	12.3 ± 1.26	36.5 ± 4.99
8. G3645	85	1.97 ± 0.48	4.50 ± 0.26	6.46 ± 0.62	23.1 ± 5.99	11.2 ± 1.67	34.3 ± 7.47
9. G5285	84	2.94 ± 0.06	6.64 ± 0.40	9.58 ± 0.37	28.1 ± 2.36	15.2 ± 2.40	43.2 ± 1.09
10. G3096	84	2.53 ± 0.09	5.69 ± 0.42	8.23 ± 0.34	27.1 ± 2.23	9.19 ± 0.51	36.3 ± 1.87
11. G22415	84	3.35 ± 0.40	8.65 ± 1.01	12.0 ± 1.26	24.3 ± 4.50	13.4 ± 2.16	37.7 ± 6.68
12. G19048	83	3.28 ± 0.10	5.18 ± 0.16	8.45 ± 0.06	24.1 ± 1.26	12.4 ± 0.95	36.4 ± 2.19
13. G19142	82	1.43 ± 0.38	4.36 ± 0.42	5.80 ± 0.72	16.6 ± 3.77	7.49 ± 1.27	24.1 ± 4.30
14. G21242	82	3.63 ± 0.96	6.77 ± 0.27	10.4 ± 1.20	27.3 ± 4.61	10.3 ± 0.55	37.7 ± 5.13
15. Ica Pijao	76	1.76 ± 0.47	4.69 ± 0.13	6.45 ± 0.44	25.1 ± 1.18	9.29 ± 1.06	36.9 ± 4.12
16. G11708	76	3.72 ± 0.45	8.44 ± 0.77	12.2 ± 1.22	29.3 ± 0.36	14.4 ± 0.57	43.7 ± 0.86
17. G11350	76	2.68 ± 0.40	4.21 ± 0.20	6.88 ± 0.33	26.3 ± 2.05	7.88 ± 1.75	34.2 ± 2.43
18. DR Kid.	76	2.80 ± 0.64	6.57 ± 0.73	9.37 ± 0.31	25.1 ± 1.18	8.78 ± 1.34	33.9 ± 1.84
19. G169	75	2.75 ± 0.19	5.57 ± 0.44	8.32 ± 0.58	34.5 ± 3.37	14.9 ± 0.27	49.5 ± 3.35
20. G2606	75	2.28 ± 0.48	4.31 ± 0.42	7.01 ± 0.65	37.3 ± 2.32	7.26 ± 1.37	38.9 ± 3.31
21. G7843	75	0.98 ± 0.20	4.39 ± 0.40	5.37 ± 0.55	20.5 ± 1.85	7.42 ± 0.56	27.9 ± 1.32
22. G16130	74	3.19 ± 0.13	6.85 ± 0.23	10.0 ± 0.12	28.5 ± 4.78	11.7 ± 1.29	40.2 ± 5.94
23. G10060	69	1.92 ± 0.48	5.88 ± 0.54	7.79 ± 1.01	23.1 ± 3.43	8.76 ± 0.76	31.9 ± 3.99
24. BAT93	69	1.46 ± 0.35	3.74 ± 0.49	5.21 ± 0.26	23.1 ± 1.33	8.76 ± 0.80	31.9 ± 1.79
25. A686	67	2.41 ± 0.29	5.13 ± 0.68	7.54 ± 0.93	26.1 ± 3.59	11.0 ± 0.48	37.1 ± 3.95
26. G87	66	2.28 ± 0.48	4.56 ± 0.35	6.84 ± 0.25	34.5 ± 3.37	9.33 ± 0.77	46.7 ± 3.08
27. Saginaw	65	2.21 ± 0.07	3.89 ± 0.41	6.10 ± 0.45	30.7 ± 5.13	6.52 ± 0.94	37.2 ± 5.94
28. G1934	63	1.57 ± 0.14	3.97 ± 0.34	5.54 ± 0.45	21.4 ± 5.82	8.38 ± 0.79	38.5 ± 5.93
29. G11656	59	1.85 ± 0.55	3.28 ± 0.11	5.13 ± 0.59	27.3 ± 3.20	4.56 ± 0.35	31.9 ± 3.24
30. G5034	59	1.82 ± 0.28	5.71 ± 0.44	7.53 ± 0.49	25.6 ± 4.09	10.0 ± 0.81	35.6 ± 4.76
31. G18249	59	1.52 ± 0.41	4.86 ± 0.62	6.38 ± 0.96	34.4 ± 6.75	12.2 ± 0.88	46.5 ± 7.61
32. Sanilac	58	1.67 ± 0.21	3.12 ± 0.09	4.79 ± 0.27	24.9 ± 2.05	5.88 ± 0.43	30.9 ± 2.23
33. G734	55	1.49 ± 0.07	4.10 ± 0.28	5.58 ± 0.29	37.7 ± 3.67	9.50 ± 0.95	47.2 ± 4.60
34. G3971	50	0.34 ± 0.07	2.76 ± 0.17	3.10 ± 0.23	24.1 ± 1.69	6.42 ± 0.66	30.5 ± 1.74
35. G11229	43	0.43 ± 0.18	2.97 ± 0.57	3.40 ± 0.76	25.3 ± 2.71	5.70 ± 0.44	30.9 ± 2.83
Tukey's $HSD_{0.05}$		1.25	1.47	2.12	12.5	3.84	13.8

ferences in their Zn concentrations in the young plant parts, but with regard to Zn content, the most Zn efficient line had 10-fold higher Zn content (Table 3). Accordingly, when ZE was calculated based solely on biomass of the young portion of the shoot, ZE varied from 9 to 90% (Figure 2).

The very close relationship between ZE and the Zn content of young parts of the shoot may indicate more efficient translocation of Zn into young parts of these genotypes. To assess the capacity of bean genotypes for Zn translocation into young tissues, the proportion of Zn in young and old parts was calculated by using the Zn content values shown in Table 3. Zn distribu-

Table 4. Percent distribution of Zn between young and old parts of shoots of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. Zinc distribution was calculated by dividing the total amount of Zn in young or old parts by the total amount of Zn in whole shoot. All genotypes are ranked according to their whole-shoot ZE

Genotype			−Zn		+Zn	
			Young parts	Old parts	Young parts	Old parts
		ZE%		%		
1.	G4449	102	36	64	67	33
2.	G11360	92	37	63	69	31
3.	G12778	86	39	61	73	27
4.	G753	86	27	73	71	29
5.	G9975	85	34	66	74	26
6.	NB585	85	36	64	83	17
7.	LRK31	85	32	68	66	34
8.	G3645	85	30	70	67	33
9.	G5285	84	31	69	65	35
10.	G3096	84	31	69	75	25
11.	G22415	84	28	72	64	36
12.	G19048	83	39	61	66	34
13.	G19142	82	25	75	69	31
14.	G21242	82	35	65	72	28
15.	Ica Pijao	76	27	73	68	25
16.	G11708	76	30	69	67	33
17.	G11350	76	39	61	77	23
18.	DR Kid.	76	30	70	74	26
19.	G169	75	33	67	70	30
20.	G2606	75	33	61	96	19
21.	G7843	75	18	82	73	27
22.	G16130	74	32	69	71	29
23.	G10060	69	25	75	72	27
24.	BAT93	69	28	72	72	27
25.	A686	67	32	68	70	30
26.	G87	66	33	67	74	20
27.	Saginaw	65	36	64	83	18
28.	G1934	63	28	72	56	22
29.	G11656	59	36	64	86	14
30.	G5034	59	24	76	72	28
31.	G18249	59	24	76	74	26
32.	Sanilac	58	35	65	81	19
33.	G734	55	27	73	80	20
34.	G3971	50	11	89	79	21
35.	G11229	43	13	87	82	18
	Tukey's $HSD_{0.05}$		11.7	12.0	13.9	8.86

tion was calculated by dividing the total Zn content in young or old parts by the total Zn content of the whole shoot (Table 4). Under Zn-deficient conditions, Zn distribution varied between 11 and 39% with a mean value of 30% for young parts and between 61 and 89% with a mean value of 70% for old parts. In the case of Zn-sufficient plants, the proportions of Zn distributed between old and young parts were reversed compared to Zn-deficient plants. When compared to Zn-inefficient genotypes (i.e., G11229, G3971), Zn

Table 5. Average seed dry matter mass, seed Zn concentration and seed Zn content of bean genotypes used in the present study. Data are presented as means ± SE, $n = 4$ replicates. All genotypes are ranked according to their whole-shoot ZE

	Genotype	ZE%	Average seed mass (g seed^{-1})	Seed Zn concentration (μg g^{-1})	Seed Zn content (μg seed^{-1})
1.	G4449	102	0.364	50.39 ± 2.44	18.34 ± 0.89
2.	G11360	92	0.360	25.85 ± 0.61	9.31 ± 0.22
3.	G12778	86	0.316	37.97 ± 4.52	12.00 ± 1.43
4.	G753	86	0.198	28.44 ± 1.07	5.63 ± 0.21
5.	G9975	85	0.404	28.41 ± 0.84	11.48 ± 0.34
6.	NB585	85	0.222	39.14 ± 4.61	8.69 ± 1.02
7.	LRK31	85	0.375	40.11 ± 1.20	15.04 ± 0.45
8.	G3645	85	0.222	41.46 ± 2.20	9.20 ± 0.49
9.	G5285	84	0.271	44.81 ± 2.49	12.14 ± 0.68
10.	G3096	84	0.259	28.91 ± 2.11	7.49 ± 0.55
11.	G22415	84	0.400	31.71 ± 1.40	12.69 ± 0.56
12.	G19048	83	0.333	25.76 ± 1.24	8.58 ± 0.41
13.	G19142	82	0.182	43.71 ± 1.69	7.96 ± 0.31
14.	G21242	82	0.366	36.83 ± 0.58	13.48 ± 0.21
15.	Ica Pijao	76	0.191	41.85 ± 4.43	7.99 ± 0.85
16.	G11708	76	0.497	23.85 ± 1.28	11.85 ± 0.64
17.	G11350	76	0.202	40.66 ± 0.63	8.21 ± 0.13
18.	DR Kidney	76	0.322	34.90 ± 1.94	11.24 ± 0.62
19.	G169	75	0.197	48.12 ± 0.43	9.48 ± 0.08
20.	G2606	75	0.189	45.62 ± 1.02	8.62 ± 0.19
21.	G7843	75	0.194	33.26 ± 1.34	6.45 ± 0.26
22.	G16130	74	0.459	24.24 ± 0.62	11.12 ± 0.28
23.	G10060	69	0.372	31.89 ± 1.33	11.86 ± 0.49
24.	BAT93	69	0.171	31.81 ± 0.33	5.44 ± 0.06
25.	A686	67	0.210	47.15 ± 0.67	9.90 ± 0.14
26.	G87	66	0.193	41.83 ± 0.12	8.07 ± 0.02
27.	Saginaw	65	0.112	56.54 ± 2.14	6.33 ± 0.24
28.	G1934	63	0.165	29.58 ± 1.11	4.88 ± 1.18
29.	G11656A	59	0.182	43.31 ± 2.50	7.88 ± 0.45
30.	G5034	59	0.371	23.98 ± 1.03	8.89 ± 0.38
31.	G18249	59	0.218	28.35 ± 2.25	6.18 ± 0.49
32.	Sanilac	58	0.118	47.39 ± 1.11	5.59 ± 0.13
33.	G734	55	0.184	41.21 ± 0.94	7.58 ± 0.17
34.	G3971	50	0.131	30.13 ± 1.15	3.95 ± 0.15
35.	G11229	43	0.147	25.96 ± 0.76	3.82 ± 0.11
	Mean		0.26 ± 0.02	36.43 ± 1.47	8.78 ± 0.58

efficient genotypes (i.e., G4449, G11360, G12778) were generally able to distribute a greater portion of the total shoot-Zn to young parts under Zn-deficient conditions (Table 4). Thus, Zn efficiency values were positively and significantly correlated with the proportion of Zn in young parts, but not in old parts of the shoot (Figure 3).

Seed size, seed-Zn concentration and Zn content

The relationship of Zn efficiency with seed-Zn concentration and Zn content is shown in Table 5 and Figure 4. Results were analyzed by analysis of variance using seed-Zn as a covariate (ANCOVA) to eliminate experimental error due to differences in seed-Zn

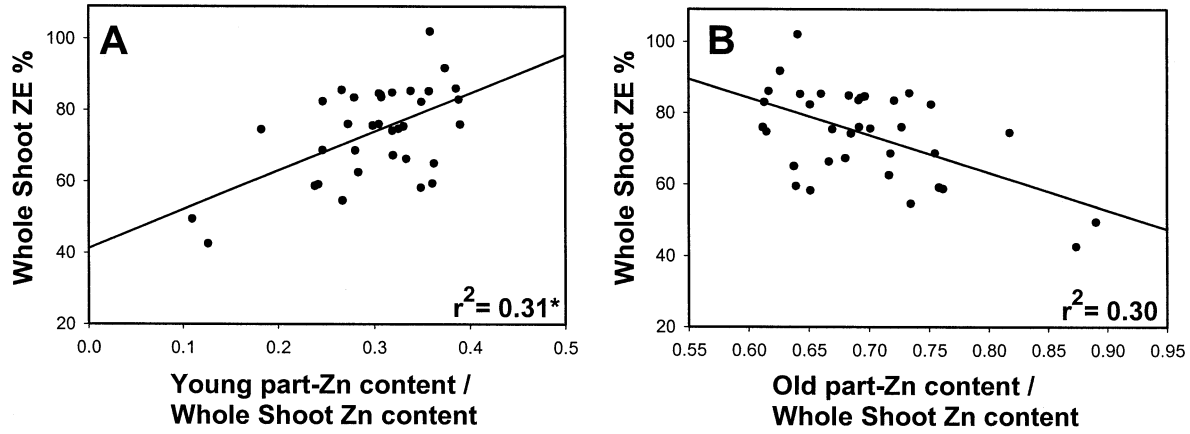

Figure 3. Correlation between % whole shoot-Zn efficiency (ZE) and the ratios of the total amount of Zn (Zn content) per young (A) or old (B) parts to the total Zn amount per whole shoot of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. *, **, and *** are statistically significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels, respectively, as determined using simple linear regression (solid line is the calculated linear regression line); r^2 = linear regression coefficient squared.

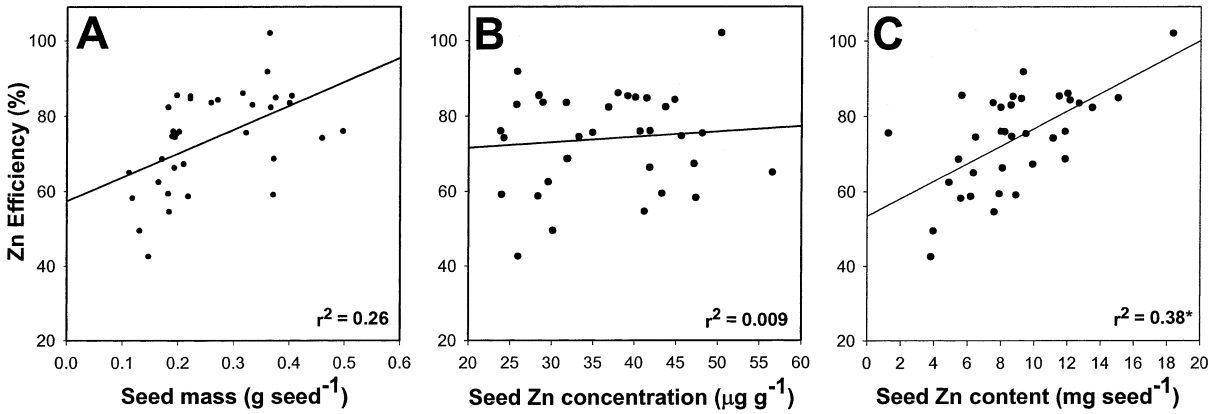

Figure 4. Correlation between % whole shoot Zn efficiency and (A) seed mass; (B) seed-Zn concentration; and C) seed Zn content of 35 bean genotypes grown for 45 d in Zn-deficient soil with (+Zn= 5 μg Zn g^{-1} soil) and without (−Zn= 0) Zn supplied. Solid line represents the calculated linear regression; r^2 = linear regression coefficient squared. * is statistically significant at $P < 0.05$ level, as determined using simple linear regression (solid line is the calculated linear regression line); r^2 = linear regression coefficient squared.

content. While seed-Zn concentration was not significantly correlated with ZE values, the correlation between seed-Zn content and ZE was moderately significant ($P \leq 0.05$; $R^2 = 0.38$). When the ZE values were adjusted to reduce the potential experimental error due to variation in seed Zn content (Figure 1), no change was observed in the Zn efficiency rankings presented in Table 1. Therefore, the variation in seed Zn content observed in the different bean genotypes had no confounding effect on our determination of ZE for these same genotypes. Genotypes greatly differed in seed mass and seed-Zn concentrations (Table 5). Only a few Zn efficient genotypes had greater seed size and seed-Zn concentrations compared to Zn-inefficient genotypes (Table 5). The genotypes having the highest (i.e. G11708, G16130) and lowest seed mass (i.e. Saginaw) and seed-Zn concentrations showed an intermediate tolerance to Zn deficiency. When all genotypes were considered, the relationship between seed-Zn concentration and whole shoot ZE was almost nil. There was a weak but not significant relationship between seed mass and the Zn efficiency trait (Figure 4).

Discussion

The present study showed a substantial genotypic variation in tolerance to Zn deficiency between 35 common bean genotypes (Table 1; Figure 1). Toler-

ance to Zn deficiency (relative growth) was based on the calculated ZE with considerations for the growth of whole shoot, as well as young and old parts of the shoot. The whole shoot dry weight-based ZE has been an extensively used parameter for assessing genotypic variation in tolerance to Zn deficiency (Rengel and Graham, 1995a; Cakmak et al., 1997; Khan et al., 1998; Torun et al., 2000; Rengel and Römheld, 2000) and also similar calculations have been used for assessing other nutrient deficiencies (Fageria and Baligar, 1999; Gourley et al., 1994). Besides whole shoot dry weight, we also examined young part- and old part-based dry weights to estimate genotypic variation in Zn efficiency. Before the development of the first trifoliate leaves there was no indication of visual Zn deficiency symptoms in −Zn plants indicating that growth of plants under −Zn treatment relied mostly on seed-Zn stores until the formation of primary leaves (old parts). Therefore, the old part-based ZE was not a suitable parameter to use in separating genotypes as to their tolerance to Zn deficiency (Figure 3). Interestingly, with the exception of one genotype, old parts of Zn-deficient plants had higher dry matter production than the plants supplied adequately with Zn (Table 1). Young part-based ZE showed a higher correlation in differentiating all bean genotypes for their tolerance to Zn deficiency compared to old part-based ZE.

To our knowledge, this is the first report of using old and young part-based ZE calculations for estimating genotypic variation in Zn efficiency. To some extent, this approach eliminates the contribution of seed-Zn to the growth of trifoliate leaves under Zn-deficient conditions. Therefore, young part-based ZE ratios appear to be a more reliable parameter for screening ZE compared to ZE calculations based on whole shoot data.

There was no correlation between the calculated ZE and Zn concentrations of plants (Figure 2). The magnitude of the genotypic variation in tissue-Zn concentrations was much smaller than the variation in ZE values (Table 2; Figure 2). However, the total amount of Zn per plant part (Zn content) was found to be closely correlated with ZE values based on whole shoot dry weight and young part dry weight (Figure 2). These results suggest that the Zn concentration of plants is not a reliable parameter in distinguishing bean genotypes for their tolerance to Zn deficiency. The total amount of Zn in young parts of plants seems to be highly useful in ranking genotypes for their ZE (Figure 2). These results are in agreement with those obtained for wheat (Rengel and Graham, 1995b; Cakmak et al., 1997; Torun et al., 2000; Hacisalihoglu et al., 2001).

The close correlation between ZE and total amount of Zn in young parts of plants leads us to the suggestion that Zn-efficient genotypes are able to translocate more Zn from roots and/or older leaves into shoot meristematic tissues. Zinc can be readily translocated from older into younger tissues via the phloem even from non-senescent old leaves, as reported for wheat (Pearson and Rengel, 1994; Erenoglu et al., 2002). The results in Table 4 generally indicate that Zn-efficient genotypes have a higher capacity to translocate Zn into young shoot parts than the Zn-inefficient genotypes. For example, the two most Zn-efficient genotypes had three-fold greater capacity to translocate Zn into young parts than the two most Zn-inefficient genotypes (Table 4). In studies with 164 wheat genotypes, Torun et al. (2000) assumed that Zn-efficient genotypes re-translocate greater amounts of Zn from older leaves into shoot meristematic tissues allowing better growth and dry matter production under Zn-deficient conditions. However, recently Erenoglu et al. (2002) reported that Zn-efficient and Zn-inefficient wheat cultivars were not different in their ability to re-translocate or distribute foliar-applied ^{65}Zn within plants cultured under Zn-deficient conditions. The role of Zn transport from roots or older leaf tissue into shoot tips and other phloem sinks during differential ZE trait expression in bean needs to be clarified in the future by conducting uptake and transport experiments using radiolabeled Zn.

As indicated above, old parts (source leaves) of plants under Zn deficiency have greater dry weight than those of Zn-sufficient plants (Table 1) which may have occurred because of the accumulation of photosynthates in these source leaves as a result of inhibited shoot tip growth (elongation) and/or reduced photosynthate export from source into sink organs via the phloem. Previous reports show that Zn deficiency results in a massive accumulation of sucrose in primary (source) leaves of bean, and resupply of Zn to deficient plants for 48 h significantly reduced sucrose accumulation in source leaves (Marschner and Cakmak, 1989). It is, therefore, very likely that maintenance of sufficient Zn concentration in older tissues is important for effective export of sucrose into meristematic tissue and other phloem sinks. One consequence of the accumulation of photosynthates in source leaves could be the formation of reactive O_2 species in the cells and photooxidative damage to the leaf chloroplasts (Cakmak, 2000). Alternatively, increases in dry

weight of older leaves under Zn-deficient conditions can be ascribed to the inhibition of new growth due to decreased concentration of phytohormones (Cakmak et al., 1989).

The seeds used in this study were collected from several different sites in the world including North and South America. Seed-Zn concentrations within those genotypes (35 common bean genotypes) studied ranged form 24 to 57 μg g^{-1} with a mean of 36 μg g^{-1} (Table 4). Interestingly, in a study with 1072 common bean accessions from CIAT's core collection, a very similar range was reported, i.e., a range between 21 and 55 μg g^{-1} with a mean of 34 μg g^{-1} (Islam et al., 2002). Seed-Zn content has been implicated in influencing plant Zn efficiency by its potential contribution to early seedling vegetative growth under low-Zn conditions (Genc et al., 2000). Zn efficiency values exhibited a moderately significant correlation with seed-Zn content ($R^2 = 0.38$; Figure 4). However, there was still a significant variation among bean genotypes after the seed-Zn effect was accounted for, and the rankings for Zn efficient and Zn inefficient bean genotypes was unchanged after the contribution of variation of seed Zn content to ZE was calculated via analysis of covariance (Figure 2). Therefore, our results suggest that for the bean genotypes tested, genotypic variation in tolerance to Zn deficiency is likely an inherited trait and not related to seed size or seed-Zn content. This is consistent with previous findings, which reported that ZE was not completely associated with Zn concentration or Zn content of the seeds in wheat (Cakmak et al., 1996; Torun et al., 2000), chickpea (Khan et al., 1998), and in *Medicago* species (Streeter et al., 2001).

Based on analysis of 35 bean genotypes, we conclude that there is substantial variation in tolerance to Zn deficiency within common bean. The results also showed a well-defined relationship between shoot dry matter and the Zn efficiency trait. In the present work, ZE calculations based on the mass of young parts and young part Zn content for shoots seem to be suitable parameters for assessment of ZE. Possibly, Zn retranslocation into young shoot parts from older tissues is linked to Zn efficiency. Further genetic and molecular physiological studies are needed to identify the gene(s) and those mechanisms controlling expression of high Zn efficiency in the common bean.

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